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VITAMIN CONTENT OF TURNIP-GREEN PLANTS IN RELATION TO GROWTH[1, 2]

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Correlations between vitamin concentration and dry weight in certain plant organs have been noted by various investigators. Wynd (8) observed that an increased concentration of carotene in the leaves of oats and rye harvested at the jointing stage was associated with increased yields of dry matter, but that concentration of ascorbic acid was not intrinsically related to yield. Also working with oat plants, Watson and Noggle (7) found that riboflavin concentration in leaves and stems, in μg per gm, was significantly correlated (5 % level) with the growth of these organs (dry weight per plant), but that correlation of riboflavin concentration with the growth of roots was not significant.

The present experiments with turnip-green plants were carried out in connection with a regional project. They were undertaken to determine the concentration of vitamins in all organs and the distribution of vitamins among organs as plant dry weight increased, and to study the relationship between the total amount of vitamin per plant and plant dry weight.

MATERIALS AND METHODS

PRODUCTION OF CROPS: Three experiments were conducted with field-grown turnip-green plants of the Seven-Top variety (*Brassica rapa* L.); plants in the first experiment were grown at Stillwater in the fall of 1950, and those in the second and third experiments were grown at Stillwater and Perkins in the spring of 1951. Seed was planted in six 200-foot rows two and one half feet apart, and a complete fertilizer was applied as a side band application to each row at the time of planting. To facilitate sampling, each row was divided into ten-foot segments.

Duplicate samples were taken at every harvest. A sample consisted of an equal number of plants from each of five segments; each segment was in a different one fifth of the field and was randomly selected from the 24 segments in that area. All segments were sampled from the same end and plants were taken in order of their occurrence. A minimum of ten plants was used for each sample and these were always the first two plants in each segment having a plant grow-

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[3] The experimental data in this paper are taken from a thesis submitted by George V. Odell in partial fulfillment of the requirements for the degree of Master of Science in Biological Chemistry in the Graduate School of the Oklahoma A. and M. College and were presented, in part, at the spring, 1952, meeting of the American Chemical Society at Milwaukee, Wis.

TABLE I

MEAN * VITAMIN CONTENT OF LEAF BLADES, MIDRIBS, AND ROOTS OF TURNIP-GREEN PLANTS (SEVEN TOP) FROM THREE SOURCES

LOCATION AND SEASON	AMOUNT OF VITAMIN PER GRAM DRY MATTER										
	ASCORBIC ACID MG			RIBOFLAVIN μG			THIAMINE μG			CAROTENE MG	
	LB †	M †	R †	LB	M	R	LB	M	R	LB	M
Stillwater, fall	10.6	7.7	8.0	28	8	7	13	3	5	0.51	0.05
Stillwater, spring	10.0	8.2	8.3	29	8	8	14	4	7	0.48	0.04
Perkins, spring	10.2	7.5	7.3	26	7	7	12	2	5	0.48	0.04
Mean ** of combined expts.	10.3	7.8	7.8	28	7.8	7	13	3	6	0.49	0.04
Standard deviation	± 1.4	± 1.4	± 1.4	± 4	± 2	± 1.4	± 4	± 1.6	± 2.9	± 0.10	± 0.04

* Mean of 24 values for duplicate field samples taken on 12 sampling days over a period of six weeks.
** Mean of 72 values.
† LB = leaf blades; M = midribs; R = roots.

ing on either side of them in the row. If more than ten plants were required, more than two were taken from each segment.

PREPARATION OF SAMPLES: Sampling began five weeks after planting and was continued for six weeks, samples being collected at 8 a.m. on two fixed days each week. Plants were pulled or dug from the row and taken at once to the laboratory where the roots were removed and the leaves separated. Leaves and roots were washed in tap water, rinsed twice in distilled water and allowed to drain. Midribs were carefully removed from the leaves and the fresh weight of leaf blades, midribs, and roots determined. Each leaf-blade, midrib, and root composite was mixed, diced, and analyzed for moisture, ascorbic acid, riboflavin, and thiamine. Carotene was determined in leaf-blade and midrib composites but analysis of root composites for this constituent was discontinued after it was established that there was no carotene present in this organ.

ANALYTICAL PROCEDURES: *Moisture:* Moisture content was determined by drying duplicate 50 gm samples of the fresh diced material to constant weight in a forced-draft oven at 70°C.

Ascorbic acid: Ascorbic acid was determined by the photometric method developed by Heinze et al (2).

Riboflavin: A modification of the procedure described by Peterson et al (4) was employed in the determination of riboflavin. Details of the method as used in this study have been described by the Southern Cooperative Group (6, pp. 138–139).

Thiamine: The procedure used for the determination of thiamine was an adaption of the method developed by Conner and Straub (1) as described by the Southern Cooperative Group (6, pp. 134–138).

Carotene: Carotene was determined by the procedure developed by Moore and Ely (3).

Methods used in the analysis of variance and in correlation and regression studies were those described by Snedecor (5).

RESULTS AND DISCUSSION

VITAMIN CONCENTRATION IN LEAF BLADES, MIDRIBS, AND ROOTS: Vitamin concentration in the organs of plants from the different sources was strikingly consistent (table I). The mean amount of each of the vitamins per gram dry matter in leaf blades did not vary significantly among the three experiments. Nor was there significant variation in the mean vitamin content of midribs and roots from the different sources, except that in one experiment the thiamine content of midribs and the riboflavin content of roots were slightly higher than in the other two experiments.

Concentration of ascorbic acid and thiamine in leaf

TABLE II

MEAN * PERCENTAGE OF THE TOTAL AMOUNT OF DRY MATTER AND VITAMIN PER PLANT CONTAINED IN THE LEAF BLADES, MIDRIBS, AND ROOTS OF TURNIP-GREEN PLANTS (SEVEN TOP) FROM THREE SOURCES

LOCATION AND SEASON	PERCENTAGE OF TOTAL AMOUNT OF CONSTITUENT IN PLANT													
	DRY MATTER			ASCORBIC ACID			RIBOFLAVIN			THIAMINE			CAROTENE	
	LB †	M †	R †	LB	M	R	LB	M	R	LB	M	R	LB	M
Stillwater, fall	62	28	10	69	23	8	86	11	3	87	7	6	96	4
Stillwater, spring	57	32	11	62	28	10	81	14	5	80	12	8	96	4
Perkins, spring	57	30	13	65	25	10	83	12	5	84	7	9	96	4
Mean ** of combined expts.	59	30	11	66	26	9	83	12	4	84	9	7	96	4
Standard deviation	± 6	± 5	± 4	± 8	± 6	± 2	± 5	± 4	± 2	± 8	± 6	± 4	± 2	± 2

* Mean of 24 values for duplicate field samples taken on 12 sampling days over a period of six weeks.
** Mean of 72 values.
† LB = leaf blade; M = midrib; R = root.

blades remained practically unchanged throughout the experimental period. With these exceptions, concentration of each of the vitamins in the different organs decreased as plant weight increased. In the combined experiments, correlation coefficients relating vitamin concentration and the dry weight of the organ per plant were negative and highly significant (1 % level) for carotene in leaf blades (−0.578), and midribs (−0.754); for riboflavin in leaf blades (−0.516), midribs (−0.607), and roots (−0.376); and for ascorbic acid in midribs (−0.522), and roots (−0.671). Thiamine concentration in the midribs was significantly (5 % level) correlated (−0.278) with the dry weight of this organ, but in the roots the correlation was not significant.

Distribution of the Whole Plant Content of Dry Matter and Vitamins among Leaf Blades, Midribs, and Roots: Distribution of the whole plant content of dry matter and vitamin among plant organs (table II) was consistent in the three experiments. The greatest proportion of each vitamin was always found in the leaf blades, which contained about 96 % of the plant carotene and 84 % of the total thiamine and riboflavin content. Distribution of ascorbic acid varied more than that of any of the other vitamins and followed the distribution of dry matter rather closely. In a period of six weeks, the percentage of total plant dry matter decreased significantly (1 % level) from 75 to 45 % in leaf blades, and increased significantly (1 % level) from 23 to 39 % in midribs, and from 5 to 15 % in roots. Similarly, the percentage of total plant ascorbic acid decreased significantly (5 % level) from 73 to 58 % in leaf blades, and increased significantly (5 % level) from 21 to 32 % in midribs, and from 6 to 10 % in roots.

Relationship between Amount of Vitamin per Plant and Plant Dry Weight: Growth, as measured by plant dry weight, was not uniform in the three experiments (table II); the mean weight of plants grown at Perkins was significantly less (1 % level) than that of plants in the other experiments. In the combined experiments, the maximum mean dry plant weight attained was 18.5 times the mean weight at the initial sampling, and the mean amount of ascorbic acid, riboflavin, thiamine, and carotene per plant increased 14-, 11-, 15-, and 9-fold, respectively.

The total amount of each vitamin per plant was positively and significantly (1 % level) correlated with the total amount of dry matter per plant in each experiment. The relationship between the total amount of vitamin and the plant dry matter was maintained regardless of plant weight, since all partial correlation coefficients determined with the age of plant held as a constant were highly significant.

The lowest partial correlation coefficients, those for carotene and riboflavin, were the result of a highly significant (1 % level), progressive decrease in the amount of each of these vitamins per gram dry matter of the whole plant (fig 1). In the combined experiments carotene decreased from 0.43 mg to 0.19 mg, and riboflavin from 26 μg to 14 μg per gm dry matter in whole plant during the six weeks' period. The amount of ascorbic acid per gm dry plant weight also decreased significantly (1 % level), but the decrease was less than in the case of carotene and riboflavin. There was no significant variation in the amount of thiamine per gm dry matter of whole plant. The mean and standard deviation of the amount of each vitamin per gram dry matter in the combined experiments were: ascorbic acid, 9 ± 1.3 mg; riboflavin, 10 ± 2.6 μg; thiamine, 9 ± 2.4 μg; and carotene, 0.3 ± 0.05 mg. Decrease in the amount of vita-

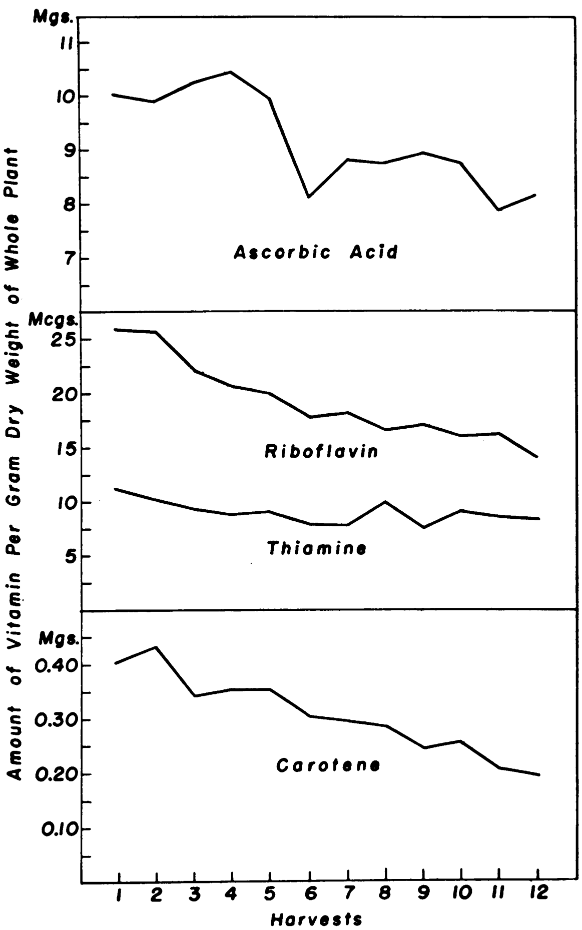

Fig. 1. Mean amount of vitamin per gram total plant dry weight in turnip-green plants (Seven Top) at twelve harvests over a period of six weeks.

min per gm dry matter of whole plant as plant weight increased was largely the result of an increasing percentage of the total plant dry matter in midribs and roots.

Regression coefficients to determine the increase in the total amount of ascorbic acid in the plant per unit increase in plant dry weight were calculated for the individual experiments, and an analysis of errors of estimate showed no significant variation among the three coefficients. Differences in the environmental conditions under which the plants were produced failed to affect the relationship between the amount of ascorbic acid in the whole plant and the dry weight of the plant. Similarly, regression coefficients for the amount of thiamine per plant on plant dry weight did not vary significantly among experiments, nor did regression coefficients for the amount of carotene per plant on plant dry weight. There was a significant variation (5 % level) among the three regression coefficients for riboflavin; the rate of increase in plant riboflavin content in the experiment at Perkins differed slightly from the rate in the other experiments.

On the assumption of a linear relationship, regression equations were derived from the data in the three experiments for the estimation of the total amount of ascorbic acid, thiamine, and carotene in the plant from plant dry weight; and an equation for the estimation of riboflavin was derived from data from the fall and spring experiments at Stillwater. These equations, the regression lines, and the experimental values from which the equations were derived are shown in figure 2.

The derived equations adequately express the relationship between the vitamin content of the whole plant and the plant dry weight when plant weight varied from 2 grams to 69 grams. Since these equations were applicable to plants produced at different locations and in different seasons, it appears probable that they may apply to turnip-green plants of the Seven Top variety regardless of their source. It is even possible that they are applicable to all varieties of turnip-green plants. Use of this type of equation provides a convenient method for the estimation of the vitamin content of the plant from plant dry weight.

Summary and Conclusions

Vitamin concentration in leaf blades, midribs, and roots of turnip-green plants from three sources appeared to be characteristic for the variety, and relatively unaffected by environmental conditions. As plant weight increased, variation in the ascorbic acid,

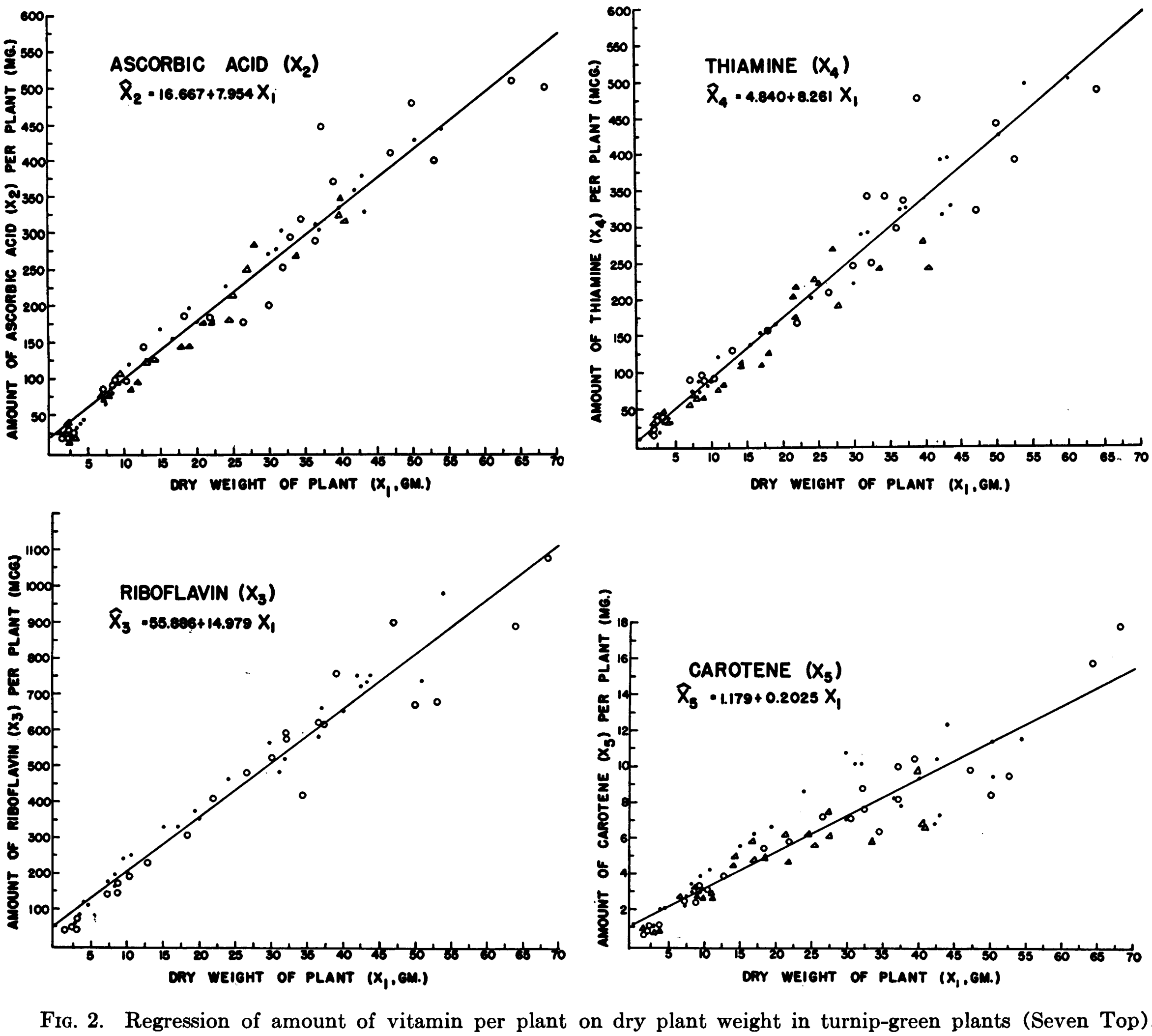

FIG. 2. Regression of amount of vitamin per plant on dry plant weight in turnip-green plants (Seven Top). Stillwater, fall = △; Stillwater, spring = ●; Perkins, spring = ○.

riboflavin, thiamine, and carotene content of leaf blades was not statistically significant over a period of six weeks. In midribs and roots, the concentration of each of the vitamins decreased as the plants grew larger and the decrease was statistically significant for each vitamin except carotene, in midribs, and thiamine, in roots.

Distribution of the whole plant content of carotene, riboflavin, and thiamine among plant organs did not change appreciably as plant dry weight increased, but there was a marked change in the distribution of ascorbic acid which rather closely followed the distribution of plant dry matter.

In all experiments the total amount of each vitamin per plant was positively and significantly correlated with the total amount of plant dry matter. Increase in the total amount of vitamin per plant per unit increase in plant dry matter was characteristic for each vitamin and was not influenced by the environmental conditions under which the plants were grown. Regression equations derived for the estimation of the amount of each of the vitamins per plant from plant dry weight were applicable to plants weighing from 2 grams to 69 grams.

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THE EFFECT OF TEMPERATURE ON THE CONVERSION OF PROTOCHLOROPHYLL TO CHLOROPHYLL A IN ETIOLATED BARLEY LEAVES [1]

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Protochlorophyll is converted to chlorophyll when dark-grown leaves containing it are illuminated, but it is not converted to chlorophyll when dissolved in organic solvents and illuminated. Conditions exist in the leaf, therefore, which facilitate this conversion. In order to analyze the mechanism of the transformation, the effects of various factors on the reaction have been examined. Among the factors examined is temperature, and it is chiefly with this factor that this paper deals.

Only fragmentary data concerning the effect of temperature on this conversion have been published. Liro (12) found that irradiation of etiolated seedlings at $-15°C$ produced chlorophyll. Scharfnagel (15) detected chlorophyll formation in dark-grown corn seedlings when they were illuminated at $-6°C$. Lubimenko (13) observed that temperature had no sensible influence on the photochemical transformation of protochlorophyll to chlorophyll. Koski and Smith (8) showed that in dark-grown barley leaves the conversion was completely inhibited by temperatures around 90°C, and that the rate of photochemical conversion was little if at all affected by changing the temperature from 5 to 18°C (6, 9, 18).

The observations that the transformation takes place at freezing temperatures, that the rate is little affected by change of temperature in the room-temperature range, and that high temperature stops the transformation suggested that a systematic investigation of the effect of a wide range of temperatures on the transformation of protochlorophyll in situ would greatly extend our understanding of this process. Accordingly, the transformation of protochlorophyll to chlorophyll a in intact dark-grown barley leaves has been studied in the temperature range from -195 to $+55°C$.

Materials and Methods

Leaf Material: Leaves from etiolated barley seedlings (*Hordeum vulgare*) were used in these experiments. The seedlings were grown in pots of sand and were watered with tap water. The temperature of the darkroom in which they were grown was about 22°C. The leaves were harvested from seedlings which had been grown from 9 to 13 days after planting. They were cut about 5 cm below the tip. During the care and handling of the plants, the only light to which they were exposed was from a flashlight screened with a dark-green cellophane filter. The exposures from the flashlight to which the leaves were subjected were ineffective for the transformation.

Analytical Methods: The percentage transformation was measured by the method employed by Koski, French, and Smith (7) in determining the action spectrum for the conversion of protochlorophyll to chlorophyll a. In brief, it consisted of grind-

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